

Plants as Bioassay Systems for Monitoring Atmospheric Pollutants

by William A. Feder*

Plant species act as natural bioindicators of atmospheric pollutants. Plants can be used as bioassay systems for monitoring atmospheric pollutants. Plant injury symptoms, altered growth and reproductive pattern, changes in yield and/or productivity, and changes in species distribution can be used singly or in combination as monitoring devices. The results must be accepted as semiquantitative, but within that constraint, air quality can be sufficiently well defined to enable the setting of air quality standards. Genetic variability of higher plant species has yielded cultivars which display a range of tolerance to gaseous and particulate atmospheric pollutants. Asexual propagation of these cultivars provides pollutant-sensitive and pollutant-tolerant plant material which can be grown on selected sites for observation. Gymnosperm and Angiosperm species as well as species of lichens and mosses have been used to establish field monitoring networks in Europe, Canada, and the United States. White pine, shade tobacco, mosses, and lichens have proven particularly useful as bioassay tools. Pollen from pollutant-sensitive and pollutant-tolerant plant cultivars has also been used as a sensitive laboratory bioassay tool for studying air quality. Epiphytic mosses are particularly efficient as monitors of particulate pollutants, especially heavy metals, some of which may act as chemical mutagens. The cost, complexity, and lack of reliability of instrumented systems for air quality monitoring make imperative the need to develop successful plant bioassay systems for monitoring air quality.

Green plants have been used as air pollution indicators for many years. (1-6). An indicator plant is one which exhibits symptomatology when exposed to phytotoxic concentrations of a pollutant or pollutant mixture. Colored pictures illustrating injury symptoms caused by various gaseous pollutants have been shown in three atlases (7-9).

Green plants can also act as indicators of air pollution by accumulating the pollutant or some detectable metabolic product of the pollutant in their tissues. Gaseous air pollutants such as hydrogen fluoride (HF) can be detected in plant tissues after exposure to fluoride-contaminated air or soils (10, 11). Sulfates in leaves after exposure of plants to SO_2 have been detected by several workers (12-16).

Particulate pollution, dusts, and aerosols containing heavy metals and the like can be detected by deposition on leafy structure of green plants, though this deposition may not cause any visible symptomatology. Here the plant acts as a collector and perhaps as an indicator of the presence of the pollutant (17-23).

Monitoring connotes something more quantitative than indicating. A plant indicator can be considered a chemical sensor which can detect the presence of a pollutant in the air. A plant monitor must be a detector, but it also must help us answer the question, how much? The U. S. Environmental Protection Agency has set standards for several gaseous and particulate pollutants. This concentration of a particular pollutant has been judged, based on the best available information, to be the maximum amount of that pollutant which can be allowed to exist in the air for a chosen time period without being injurious to plants or animals. This would be the dose which, as long as not exceeded, would not have deleterious effects on plants or animals. While the criteria for arriving at these standards are less than perfect and much is unclear about how edaphic and environmental factors affect sensitivity of plants to pollutants, still the standards represent a target concentration which, if achieved, should protect living organisms against a variety of known pollutants. The concept of this type of standard is similar to that used in attempting to protect living organisms against various types of harmful radiation. Knowing the radiation standards, various

*Suburban Experiment Station, University of Massachusetts, 240 Bever Street, Waltham, Massachusetts 02154.

workers have attempted to learn how plants at the organismic or the cell level or, indeed, the molecular level respond to various radiation doses and have then attempted to utilize this information to measure ambient radiation using the plant as a biological monitor. The change in color, and other characteristics of the stamen hair of *Tradescantia* cultivar, is a response system which has been adapted to act as a monitor for ambient radiation. This work has been discussed in several papers at this Workshop, detailed in a series of papers by Ichikawa and Sparrow (24-26).

There are three general ways in which plants could be, or have been used as bioassay systems for monitoring air pollutants. The first has been to attempt to integrate the degree of pollutant-incited injury with known ambient pollutant concentrations. The second has been to use the plant as a living collector. The third has been to attempt to measure the amount of pollutant, or pollutant-related metabolite which appears in the tissues after exposure to the pollutant and to attempt to relate that quantity with the amount of pollutant in the ambient air. This paper will discuss each of the above categories separately, though there may be instances when more than one of the approaches mentioned might be utilized with the same plant bioassay system. The pollutants to be considered will be ozone, sulfur dioxide, hydrogen fluoride, and heavy metal particulates. There are, of course, hundreds, perhaps thousands of other pollutants including, according to Pitts (27), some with possible mutagenic properties present in aerosols found in the South Coast air basin of southern California. However, I will confine this discussion to the ones listed above.

Pollutant-Incited Injury

Normal genetic variability within populations of many commercially important herbaceous and woody plant species has produced cultivars of many species which show a range of tolerance levels to one or several air pollutants. Among the most useful as possible monitoring tools are several cultivars of *Nicotiana tabacum* L. These are Bel-B, ozone-resistant; Bel-C, ozone-sensitive; and Bel-W3, ozone supersensitive, which were identified as indicators for ozone and PAN, another phytotoxic oxidant (28, 29).

Tobacco (*Nicotiana tabacum* L.), used extensively in studying the photochemical oxidant complex (30), has proven to be an excellent monitor for several reasons. It produces new leaves continuously during the growing season. Leaves of different maturity differ in sensitivity and leaves are uni-

formly sensitive at a given stage of growth. New injury is easily separated visually from old injury. A Bel-W3 plant shows characteristic, easily identifiable, and quite specific symptoms of oxidant injury. The so-called 'flecking' is made up of numerous small lesions, primarily on the upper leaf surface of fully expanded leaves. Bifacial lesions are common on the very sensitive cultivars like Bel-W3. The lesion begins as a water soaked area. The area within the lesion becomes necrotic, turns dark, and within 48-72 hr turns light gray or tan as the tissue dries. Lesion size depends on the ozone dosage, the cultivar, and environmental factors, but typical lesions are less than 2 mm in diameter and rounded or irregular in outline. On a sensitive cultivar like Bel-W3, the lesion may be as large as 6 mm in diameter.

The flecking of ozone-sensitive Bel-W3 is not only specific for a single pollutant, ozone, but also quite clearly reflects the ambient concentration of that pollutant. That is, the plant responds to increasing doses of ambient ozone by developing more flecks or lesions. The threshold dose at which ozone causes injury to Bel-W3 tobacco leaves is about 0.05 ppm for 4 hr (28). This threshold may vary with the sensitivity of the plant, which is, in turn, affected by soil type, soil moisture stress, light, temperature, and nutrient level of the soil (31). However, growing tobacco using standard cultural techniques (32) 0.05 ppm ozone for 4 hr, seems to be a good baseline figure to use in interpreting the response to Bel-W3 tobacco to air quality. Based on a series of photographs illustrating varying degrees of leaf injury, an injury rating scale was developed using a visual estimate of the per cent leaf surface covered by lesions (flecks) (32). This injury index was used to measure the extent of injury after exposing Bel-W3 tobacco plants to differing doses of ozone. The response was found to be nonlinear (34). However, a linear dosage response curve was developed by arranging large numbers of Bel-W3 plants on a grid laid out over a ¼ mile radius and exposing the plants to ambient ozone levels over a period of a month (35). This grid was adapted to a large scale study of ozone transport over the open ocean to Nantucket Island. The entire island was considered a single site and tobacco plants were placed in small groups at selected loci throughout the site to create a large X pattern. This assured that plants would be in position to catch the wind from all directions of the compass and would, therefore, serve to monitor the ambient ozone concentrations regardless of which wind trajectory brought the ozone-polluted air mass to the island, which itself is essentially ozone-free. By continuous instrument monitoring of ozone, as well as by tobacco injury

indexing, it was found that ozone was passing over the island on southwest wind patterns and that the cumulative ozone load was positively correlated with the tobacco injury index. The work, carried out over two consecutive summers, clearly indicated that ozone was being transported on air masses that were originating somewhere between Washington, D. C. and New York City and carrying out over the open ocean for several hundred kilometers before impacting on Nantucket. Ozone levels over Nantucket were considerably lower during the second summer, and this difference was recorded, both electronically and biologically, showing that the plants could actually perform reliably as bioassay tools to monitor air quality. It was possible to monitor a large land mass with a single instrument by using biological monitors (36).

This laboratory is attempting to develop dosage/response slopes for Bel-W3, the ozone-sensitive tobacco cultivar, and for Bel-B, the ozone-resistant tobacco cultivar. The pitch of the slopes relative to one another when plants of both cultivars are exposed to the same air environment should reflect the air quality in terms of ozone. If the injury index slopes are steep for both cultivars, the air quality would be judged to be poor; i.e., high ozone concentrations; if the slope of Bel-W3 is steep, and that of the Bel-B more gentle, then air quality would be judged to be moderate. If the Bel-W3 slope is less steep, and the Bel-B slope is flat, air quality would be judged fair and, finally, if both slopes were flat, air quality in terms of ozone would be judged good. This type of plant bioassay system would be inexpensive, adaptable, and would give a reasonable evaluation of air quality in terms of ambient ozone.

Annual blue grass and *Petunia* spp. (37), tobacco (38), and pinto bean (39), have all been used for monitoring photochemical oxidants, especially ozone. However, all these plant materials are herbaceous and can only be used during the normal summer growing season, except in areas like Florida or Southern California where crops are grown on a year-round basis. For this reason, it is important to have another type of plant material which can survive a northern winter. Also, since many pollutants continue to be generated during the winter as well as the summer, it is useful to have a monitor which can respond regardless of the weather conditions.

Eastern white pine, *Pinus strobus* L. responds to both sulfur dioxide and ozone (40). A disease known as chlorotic dwarf of eastern white pine is probably caused by the interaction of ozone and sulfur dioxide. Differential susceptibility of species and cultivars of white pine to injury by ozone has been described (41-43). Ozone-sensitivity in white

pine varies greatly among individual trees in a population. By selection and asexual propagation clonal material of white pine with differing ozone-susceptibilities has been developed (41). These selections are being propagated and perpetuated by the U. S. Forest Service Laboratory in Delaware, Ohio, and some of their progeny are also planted and maintained by the Tennessee Valley Authority and by the Virginia Polytechnic Institute in Blacksburg, Va.

Here then, is a bioassay tool for long-term monitoring of several air pollutants. The effects of ozone on eastern white pine are described as follows (44). The great majority of white pines are relatively tolerant of ozone, but a very small percentage are so sensitive that they are severely injured each year, seldom attaining great age or size. They are further characterized by dwarfed and conspicuously burned and/or chlorotic foliage that is retained only a single year instead of the normal 27 months. Trees of intermediate sensitivity usually display near normal growth rates and needle sizes, but their needles may appear somewhat chlorotic and/or spotted with chlorotic spots, tending also to be cast prematurely. The foliage of such trees may display conspicuous tipburn in some seasons, but seldom in two successive years. Acute injuries are caused by exposure of sensitive foliage to unusually high concentrations of ozone for a few hours or less, while chronic injury is the result of daily exposure to a lower concentration of ozone, insufficient to cause distinct lesions but sufficient to cause some intensification of existing symptoms and presumably to reduce the photosynthetic efficiency of sensitive trees. An inconspicuous flecking, visible only under magnification, appears to radiate from individual stomata located in the sensitive zone 10-20 mm from the needle sheath. In microscopic view, the flecks are caused by the collapse of individual mesophyll cells adjacent to the stomata. The collapse of additional cells may occur if the injury is moderate, leading to the external appearance of yellowish to pinkish spots on the stomatal surfaces of the needles; if the injury is still more severe, the spots may become obviously necrotic and enlarge to form dead bands that widen toward the needle tips, causing tipburn. High temperature, bright sunlight, and rapid air movement appear to accentuate symptom development. Moisture on pine foliage enhances the effect of the toxicant and causes "water spots" different from the lesions induced on dry foliage.

In conifers exhibiting typical sulfur dioxide injury, the markings usually begin at the needle tip and extend toward the base. The degree of extension is related to the severity of the exposure. When

successive exposures occur a distinct banding pattern often appears. With moderate exposure to sulfur dioxide, the older needles of conifers tend to become chlorotic and are shed prematurely. With exposure to larger dosages, needles develop a water-soaked appearance which soon changes into reddish-brown necrosis of the tip. The necrotic pattern may first appear as bands around the needle with the terminal portion later turning a reddish brown. Seldom is a completely green needle observed in an affected fascicle. The middle-aged needles exhibit the most necrosis, but the older needles are cast first. Expanding needles are rarely injured. Needles tend to persist on young branches; this results in an absence of needles at the base of branches and at the bottom of the tree. This tendency for older needles to be shed prematurely results in an increasing needle shortage. Such trees make limited growth and may die prematurely.

A trained worker can distinguish between ozone and sulfur dioxide injury to white pine. However, since ozone is known to occur regularly in rural and remote forested areas, it is quite likely that both ozone and sulfur dioxide may be present in ambient air, and the white pine will respond to both pollutants and this integrated response can be utilized as the bioassay. The pine tree responds to ozone and/or sulfur dioxide pollutant stress in three ways: (a) development of injury symptoms as described above, (b) the accumulation of excess sulfate in the needles in the case of exposure to chronic ambient sulfur dioxide in the atmosphere (44), and (c) changes in its rate of growth and productivity (45). Following a simple system devised in West Germany (45) a series of test stations can be strategically located at different distances upwind and downwind from a point source and planted with groups of white pine trees, carefully selected for their resistance or susceptibility to one or both pollutants.

These trees, once in place, can be observed for visual symptoms, can be analyzed for sulfates in the needles, and can be subjected to periodic growth measurements including needle length, trunk diameter, number and length of new branches, total height, number and size of female cones, and number of seeds per cone. If care is taken to plant in areas having similar soil types and exposure to sun and wind, and similar elevations above sea level, the data collected should be a reasonable measure of the pollutant stress. Summer monitoring with plant materials which develop symptoms which are readily recognizable as being caused by one or the other of these pollutants such as tobacco or Pinto bean for ozone and alfalfa or clover for sulfur dioxide can assure the investigator, without instrumentation, as to which pollutant or pollutant

combination he is dealing with.

There is little doubt that individuals in a plant population respond differently to stresses set up by any of these pollutants (46). The demonstrated differences in response to ozone of tobacco, bean, tomato, petunia, and white pine individuals confirm this statement. Of considerable interest is the fact that pollen from a population of an ozone-susceptible tobacco cultivar is also ozone-susceptible when germinated *in vitro* in the presence of low levels of the toxicant. Pollen from the population of an ozone-resistant tobacco cultivar is ozone-resistant when germinated in the presence of the same toxicant concentration (47, 48). Similarly pollen germination and tube growth in tomato and cucumber was depressed by hydrogen fluoride (49, 50) and by sulfur dioxide (51). There is no evidence that microsporophytic development is hampered by the presence in the atmosphere of a pollutant like ozone, but there is good evidence that at high levels of ozone, pollen germination is inhibited or prevented, and at lower ozone concentrations pollen tube elongation is markedly inhibited (52, 53). These data coupled with other data (54-60) relating to the depression of fruit yield in many commercial crop plants after exposure to ozone, sulfur dioxide or hydrogen fluoride would appear to implicate the plant reproductive system as a possible target for pollutants. The effects of pollutants on plant growth and development coupled with their effect on plant reproductive system would seem to be best reflected in yield, measured in terms of fruit set, fruit numbers, and fruit size. Whether applied to grapes, pine cones, or beans, these three criteria, integrated as yield, are probably the best tool to use in monitoring atmospheric pollutants with higher plants. If the experimental setup is planned to yield good statistical information and the soil types and other edaphic and environmental conditions are comparable among sites, then how a group of plants reproduces would be a good measure of the quality of the air in which the plants are growing. Several years of annual plantings on the same sites coupled with long-term observations on woody plant materials on the same sites would yield a record of plant performance which would indicate that air quality is, or is not, a problem in that general area. Should there be a problem which can only be attributed to air quality, then instruments can be brought into the area to ascertain which pollutants are present and at what concentrations.

Plants as Living Collectors

From the point of view of this workshop it would seem that the collection of particulates, especially heavy metals, would be of considerable interest and

concern since many of these metals may be mutagenic. While mosses and lichens are not strictly "higher" plants, I trust it is the sense of the workshop to mean plants other than Prokaryotes when using the term "higher" plant.

Lichens and mosses are eukaryote plants which are conspicuous and require no special laboratory techniques for handling. Certain mosses such as *Hypnum cupressiforme* are capable of taking up heavy metals such as zinc, lead, cadmium, nickel, copper, and magnesium. The metals are not only passively caught in the moss leaves, but are also absorbed by the plant and accumulated. Thus, by collecting the moss plants, drying and weighing them, and then subjecting the dried weighed samples to chemical analysis, the amount of metal uptake can be calculated (18).

This technique was used (18) to establish the heavy metal content of the atmosphere in the Lower Swansea Valley. Transects upwind and downwind from the industrial town of Swansea and Neath and the rural area of the Gower Peninsula were used to compare levels of metal particulates in the air. In the first phase, moss samples growing on the trunks of *Quercus petraea* were collected for assay. This oak species was chosen because it lacked water tracks which might have transported water and heavy metals from the leaf canopy down the trunk into the mosses. Data obtained in this manner gave metal levels which had accumulated up to the date of sampling. They reflected from which direction the metals were coming and how much had arrived, but failed to give the rate of accumulation. To accomplish this, a second phase was instituted in which large logs covered with *Hypnum* moss were removed from an uncontaminated site and placed at several sites downwind from Swansea. By sampling the moss plants before removal to the new sites, exposing them on site for 8 weeks and then sampling again the rate at which metals were accumulating at several sites could be ascertained. In a third phase, the moss was removed from an uncontaminated site, washed, and dried, and 1.5 g of the washed, dried moss was put into nylon bags and the bags hung in trees at selected sites. By comparing the chemical analysis of the moss when it was first collected and after the bags had been hanging for several weeks, the rate of heavy metal accumulation was established. It was found that the moss was capable of not only trapping but of absorbing large amounts of lead, zinc and cadmium. After 4 weeks exposure in one site the *Hypnum* samples contained 11,611, 7,166, and 653 ppm of lead, zinc and cadmium respectively. The authors feel that there may be some sort of ion-exchange process taking place to account for these large amounts of

metal uptake. In any case, the moss bag was highly effective in the hands of Goodman and Roberts (8) and was subsequently successfully adopted by other workers in England, Europe, and Scandinavia to measure ambient levels of heavy metals (22, 61-67).

It is of more than passing interest that there was a "moss desert" for several miles downwind to the north of Neath and Swansea (18). This seemed to imply that epiphytic mosses like *Hypnum* might also be adapted as a bioassay system for monitoring sulfur dioxide. Since mosses like *Hypnum* are epiphytic, it should be possible to wash them with deionized or distilled water, establish the metal content of these washed samples and to then hang out nylon bags containing the washed living moss at selected sites (68). The moss should be able to survive with ambient rainfall and thus act as a living collector and monitor of sulfur dioxide and heavy metals in the ambient air. Mosses undoubtedly are also capable of fluoride uptake and they may also be susceptible to other gaseous pollutants like ozone, but there are no data concerning these possible pollutant/moss interactions. The bag of epiphytic moss would seem the ideal monitoring tool. Moss is plentiful, and there is no reason why, after steps have been taken to find which mosses are most effective and possess the best survival characteristics, such mosses could not be grown on a large scale under controlled conditions. Care can be taken to keep the plants from fruiting to insure genetic homogeneity in the populations produced. Standardization of washing and analyzing techniques would insure reliability and reproducibility of results and would allow many workers to take advantage of a standardized simple inexpensive technique. The fact that the same bioassay system could be used by many workers would go a long way towards reducing the current confusion in attempting to interpret results of different workers using different systems for monitoring heavy metals.

Good success has also been achieved by using various lichen species as monitors of sulfur dioxide pollution (69-73).

Several workers have noted so-called "lichen deserts" in the vicinity of sources of sulfur dioxide (74, 75). It was found that in some cases levels of sulfur dioxide were high enough to kill all lichen species, while in areas with lower levels of sulfur dioxide certain lichen species thrived, while others were unable to survive. By a combination of instrument monitoring and observation of lichen survival and speciation, it was possible to correlate lichen performance with sulfur dioxide concentrations in the ambient air (76-79). Using these data,

sampling networks could be set up covering large areas of land (80–83). It was simply necessary to record the presence or absence, and the vigor of several different lichen species upwind and downwind of a suspected source of sulfur dioxide. The presence or absence and the vigor, measured by the color and circumference of the lichen body, would tell the investigator whether or not sulfur dioxide was present and at approximately what levels (84–86). In this manner, extensive surveys were made of London, England (73), other parts of England (74, 75, 79), Ireland (70, 80), France (83), Canada (78, 81, 86), Sweden (80), and Long Island, U.S.A. (87). The advantages of this bioassay system are its low cost, simplicity, and reliability. No equipment is required aside from pencil, measuring tape, and notebook. Care must be taken to carefully mark the sites so that the same areas can be sampled in succeeding years and the observer must know his lichens.

Pollutant Uptake as a Measurement of Ambient Pollution Levels

While ozone cannot be detected in plant tissues after exposure, it is possible to detect both sulfur dioxide, as sulfates and hydrogen fluoride as F in plant tissues. This opens the possibility of using plants as bioassays for monitoring these two gases, by analyzing plant tissues for increased sulfate or fluoride ions, and relating these increases to levels of the two gases in the atmosphere in which the plants are growing. The relationship is not that simple. Fluoride can accumulate in plant tissues until the plant dies from fluoride intoxication. The levels accumulated by the plant rise far above the ambient level, thus giving a false impression as to the actual concentration of fluoride in the air. Clover exposed for 12 days to $0.85 \mu\text{g HF/m}^3$ air stored 8.7 mg F/100 g dry weight of tissue. At $1.1 \mu\text{g HF/m}^3$ air the storage level rose to 29 mg F in 100 g of tissue (88, 89). In work with alfalfa and orchard grass it was found to be more reliable to measure fluoride in the leaf tissues than in the air in order to protect livestock against fluoride poisoning (90).

A different type of difficulty is encountered when dealing with sulfur dioxide and sulfate accumulation in plants. Here the plant is able to metabolize modest amounts of S, so that accumulation may not be evident in leafy tissues unless the plant is stressed with an acute dose of sulfur dioxide which it is unable to detoxify. Studies with S^{35} -labeled sulfur dioxide indicated that uptake by tomato plants was very rapid and that translocation of S transported that material throughout the plant, including the

roots from which it could be eliminated (91). If the level of S was increased during a fumigation, it slowly decreased after fumigation, and if the plant was not overloaded the S absorbed was simply metabolized by the plant.

It would seem that with adequate care given to understanding the relationship between atmospheric HF and a particular plant species or cultivar, like *Gladiolus* cv Snow Princess, a bioassay could be worked out by using tissue analysis to estimate the ambient HF level. One could use herbaceous or woody plants as monitoring tools, but a genetically uniform population of each plant material would have to be available, and the relationship between fluoride uptake and atmospheric fluoride would have to be well understood for population.

As stated earlier (12–14) some success was achieved in characterizing the ambient sulfur dioxide level by chemical analysis of sulfate in conifer needles. It might be possible to monitor sulfur dioxide if sampling and analysis are done on a weekly basis. The results would be in the form of fluctuating levels of sulfate in the tissues and it would be necessary to integrate long term trends in these fluctuations. This methodology is necessary because of the ability of the plant to detoxify, utilize, and/or eliminate sulfur from its tissues. However, the methodology appears to be very labor-intensive, very time-consuming, and therefore, to have no real advantage over instrumentation except for the fact that it is difficult to keep electronically operated instruments "on-line" in remote areas.

In my opinion, remote areas could best be monitored by using carefully bred or selected trees, both deciduous and coniferous, which have known response thresholds, above which they display both injury symptoms and changes in growth rate and productivity (92, 93). If these plants were selected for specific visible markers which showed that they had reacted to a specific concentration of a pollutant or pollutant mix, it would be possible to detect these changes by remote-sensing, thus allowing the mapping of large areas with little manpower and with relatively little expended time. The remote sensing could then be followed up, where necessary, by ground crews which could establish whether changes in growth pattern and yield had actually occurred in areas where remote sensing had recorded apparent tree injury.

Probably the best answer to the monitoring problem, given the current state of the art would be to utilize several techniques, either at the same time or in series. For long-term studies in remote areas, tree selections should be planted in carefully sited gardens or as forests, so that they may be used as

monitors for large areas over long time periods. Wooded or forested areas, which are known to be impacted by a pollution source, and which are readily accessible, would better be handled by using lichens or the moss bag techniques described earlier. Open country, such as farm land and urban areas would be best studied by the use of herbaceous monitoring plants like tobacco, bean, soybean, petunia, etc., where the plant/pollutant interaction is well understood and injury/concentration ratios have been worked out.

Our weakest link is the lack of understanding of the genetics of pollution tolerance in green plants.

More research should be encouraged to explore the genetics of pollution sensitivity and tolerance in woody plants, and indeed in all plant materials (94-96). Cataloging differences in tolerance among individuals in a population or differences among species is useful, but does not go far enough (97, 98). If we understood more of the genetic mechanisms involved in resistance or susceptibility it might be possible to develop isolines in which pollution tolerance was the sole genetic difference between two plant populations of the same species or cultivar. For example, it has been shown that a single gene controls ozone resistance in *Allium sepa* L. and that resistance is related to stomatal behavior (99). Several workers have demonstrated the importance of stomatal behavior in the way plants react to sulfur dioxide and much is now known about genetic control of stomatal development (100-102). The more refined the plant response to a pollutant, the more likelihood of success in using that plant as bioassay for monitoring pollution.

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